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TECHNICAL NOTE

# A simple method to determine millimolar concentrations of sodium in nanoliter samples

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**A simple method to determine millimolar concentrations of sodium in nanoliter samples.** Measurement of sodium in the nanoliter samples obtained from *in vitro* studies of isolated, perfused nephron segments has traditionally been associated with time-consuming sample preparation and/or expensive instrumentation. A new instrument is described which can measure picomole quantities of sodium without the need for special processing. The instrument is based on the continuous flow devices developed by Vurek and a sodium-sensitive glass detector. The response to varying concentrations of NaCl was logarithmic as predicted from the Nernst equation. The response to 50 mM NaCl was 22.1 ± 0.2 mV; 100 mM, 29.9 ± 0.2 mV; 150 mM, 35.2 ± 0.2 mV; and 200 mM, 39.3 ± 0.6 mV (mean ± SD). The slope of the standard curve was 27.8 mV/log unit change in sodium concentration. Potassium, calcium and ammonium, possible interfering ions, had no effect on the response to 100 mM NaCl. When the response to 100 mM NaCl was measured in buffered solutions of varying pH, it was 29.1 ± 0.2 mV at pH 6.21, 28.9 ± 0.4 mV at pH 7.04 and 28.7 ± 0.4 mV at pH 8.54, indicating that pH did not alter the response to 100 mM NaCl. By treating the detector with NaOH and HCl, the response to 50, 100 and 150 mM improved to the point that the slope of the standard curve was 51.2 mV/log unit change in sodium concentration. The calculated resolution after treatment was 1.0 mM in the 50 to 100 mM range and 1.3 mM in the 100 to 150 mM range using a 10.9 nl pipet. Samples could be injected every four minutes, permitting measurements to be made in "real time" during an experiment.

In the past, measurements of picomole quantities of sodium have required either atomic absorption spectrophotometry in the furnace mode [1, 2], electron microprobe analysis [3–6], helium-glow photometry [7, 8] or proprietary chromogenic ionophores [9]. The first two techniques require extensive sample handling and preparation before measurements can be made. More recently, a method was described using chromogenic ionophores to measure sodium content in the picomole range using a continuous-flow ultramicro colorimeter; however, this method requires proprietary compounds, elevated temperatures in order to achieve maximum sensitivity, and incubation of the sample with the chromogen for up to 10 minutes [9].

The continuous-flow devices developed by Vurek [10–12] have relied on fluorometric or colorimetric detectors. However, a continuous-flow instrument was developed with a potentiometric detector based on a potassium-sensitive electrode [13]. In theory, it should be possible to construct an analogous device to measure picomole quantities of sodium based on the use of sodium-sensitive glass and these ultramicro continuous-flow devices.

This report describes a new instrument for measurement of millimolar concentrations of sodium in nanoliter volumes based on the aforementioned technology. Measurements are highly reproducible, with coefficients of variation of 1.5%. Resolution may be as fine as 1 to 2 mM within the physiological range of sodium concentrations.

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## Methods

### *Instrumentation*

Figure 1 shows a schematic diagram of the device. The instrument consists of a dissecting microscope with pipet holder, reservoir, injection port, sodium-sensitive glass detector, reference electrode, syringe pump, electrometer and chart recorder.

The dissecting microscope was arranged so that it could view the horizontal aspect of the injection port. A pipet holder was attached to the head of the dissecting microscope, allowing the worker to view the pipet and maneuver it to the injection port using the focus controls.

The reservoir was simply a Pasteur pipet. The injection port was fabricated from borosilicate glass tubing with an inside diameter of 1.02 mm and an outside diameter of 1.19 mm. After sealing one end of the tubing, the open end was attached to a syringe so that the internal pressure could be increased. The tubing was mounted in a microforge and a small area heated; as it melted, pressure was applied via the syringe, blowing a hole through the wall of the glass. Once the port was created, the sealed end was cut off and this end drawn to an inside diameter of 0.3 mm.

The "detector," the site where the stream encounters the sodium-sensitive glass, was made by drawing sodium-sensitive glass capillary tubing (Microelectrodes, Inc., New Londonderry, New Hampshire, USA) to an inside diameter of approximately 0.3 mm. Crystalline AgCl was melted onto the center of the capillary, such that approximately 1 mm of the sodium-sensitive glass was encased in AgCl. The AgCl was then reheated to its melting point and a 75 μm Ag wire inserted into the AgCl bead to form a "solid state" sodium electrode. The capillary was then trimmed so that the total length was approximately 5 mm (Fig. 1).

The reference electrode was created by melting a hole

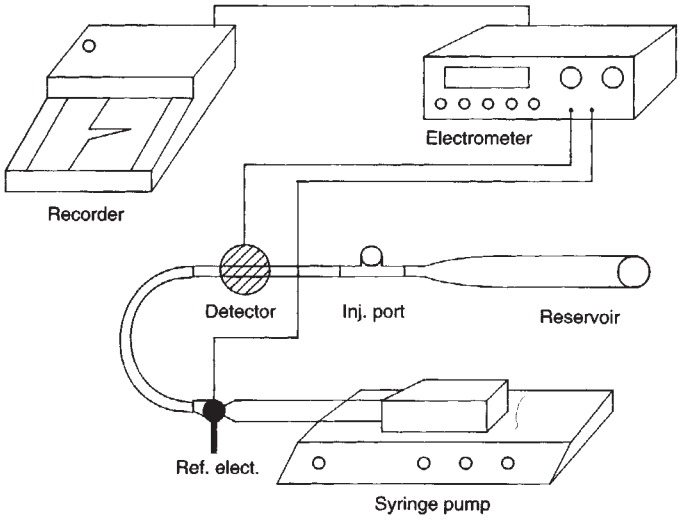


**Fig. 1.** *Schematic diagram of the potentiometric instrument.* The AgCl bead of the detector is approximately 1 mm long and encases the entire circumference of the sodium-sensitive glass.

through the body of a 27 gauge plastic-bodied needle. A pellet of AgCl was melted onto a 75 $\mu$m Ag wire and placed inside the body of the needle with the wire extending through the hole to the outside. The hole was then sealed with epoxy resin.

The reservoir was connected to the injection port with Tygon tubing. The injection port was connected to the detector with 10 cm of Silastic tubing (i.d., 0.3 mm). The Silastic tubing from the outlet of the detector was connected to the needle containing the Ag/AgCl reference electrode. The needle was mounted on a three-way stopcock which in turn was mounted on a 2500 $\mu$l gas-tight syringe in a syringe pump (Sage 352, Cambridge, Massachusetts, USA). Once this was completed, the detector and reference electrode were connected to an electrometer with an input impedance of $10^{14}$ ohms (A-M Systems Neuroprobe Amplifier Model 1600, Everett, Washington, USA). The easiest means of monitoring the response of the electrode was to record the output of the electrometer on a chart recorder (Kipp and Zonen BD 40, Delft, Holland), although the signal could also be digitized.

The electrometer probe and detector were electrically protected with a grounded shield. The dissecting microscope was also grounded to minimize electrical noise when samples were injected. The entire apparatus was enclosed in a Faraday cage which was grounded to the same point as the microscope and probe shield.

### *Operation*

To operate the instrument, it is first necessary to fill the reservoir and lines with 100 mM $MgCl_2$, making sure no air bubbles are present. This solution approximates the density of mammalian physiological saline, and $Mg^{+2}$ does not interfere with the measurement of sodium. (Matching the densities of the reservoir solution and the samples prevents injected samples from either sinking to the bottom or floating to the top of the flowing stream and adversely affecting reproducibility.) To accomplish this, degassed 100 mM $MgCl_2$ is backflushed through the stopcock. The 100 mM $MgCl_2$ solution should be degassed by heating while under a vacuum. Degassing the solution improperly will cause bubbles to form in the lines, which will affect the sensitivity of the instrument by altering solution flow. Once the lines are filled, the syringe pump can be set to draw fluid through the instrument at the rate which allows the greatest sensitivity (approximately 2 $\mu$l/min in this study). Nanoliter volumes of the sample are injected into the port, which is viewed through the dissecting microscope. Samples are injected in less than two seconds. The $MgCl_2$ solution is continually drawn through the instrument, carrying injected samples to the sodium-sensitive glass detector. It also acts as a conductor between the sodium-sensitive glass detector and the Ag/AgCl reference electrode.

### *Analysis*

To analyze the sodium concentration in a sample, a standard curve which brackets the sodium concentrations of unknowns is first generated from peak heights, which represent the change in millivolts from baseline, on the chart recorder. Peak heights are determined by measuring from the zenith to the baseline at the center of the peak. The central baseline is interpolated from points just before and after the peak. Sodium concentration in the unknown sample is determined by measuring its peak height and interpolating from the standard curve.

### *Results*

Multiple injections of four different sodium concentrations ranging from 50 to 200 mM are shown in Figure 2A. The mean peak heights for 50, 100, 150 and 200 mM NaCl were 22.1 ± 0.2, 29.9 ± 0.2, 35.2 ± 0.2 and 39.3 ± 0.6 mV (mean ± SD). The assay was highly reproducible, with coefficients of variation (standard deviation/mean) of 0.9% for 50 mM, 0.7% for 100 mM, 0.6% for 150 mM and 1.6% for 200 mM. In Figure 2B the data are plotted as a function of the logarithm of the sodium concentration: this yielded a straight line whose equation was $Y = 27.8X - 25.2$ with a correlation coefficient of 0.998. Thus, the response of the detector to changes in sodium concentration can be assumed to be logarithmic.

Table 1 and Figures 3 and 4 illustrate the effects of other cations that might be encountered or might vary in an experimental setting. For the instrument to be useful, it must be highly selective for sodium. The manufacturer reports that the sodium-sensitive glass has a selectivity for sodium over potassium of 1000 to 1 and a selectivity for sodium over ammonium of 3000 to 1. The useful pH range is stated to be 3 to 12.

Table 1 compares mean peak heights of samples containing 100 mM NaCl plus 5 mM ammonium or potassium to samples containing 100 mM NaCl alone. Ammonium and potassium are probably the ions of primary concern with respect to interference when measuring the sodium concentration of samples obtained from isolated, perfused renal tubule experiments. The mean peak height of three samples of 100 mM NaCl alone was 29.8 ± 0.4 mV. When the sample contained 5 mM $NH_4Cl$ in addition to 100 mM NaCl, the mean peak height was 29.9 ± 0.4 mV. Similarly, the response to 100 mM NaCl alone did not differ from the response to 100 mM NaCl plus 5 mM K.

To examine whether the presence or absence of calcium had any effect on the ability to detect sodium, the response to samples containing either 100 mM NaCl or 100 mM NaCl plus 5

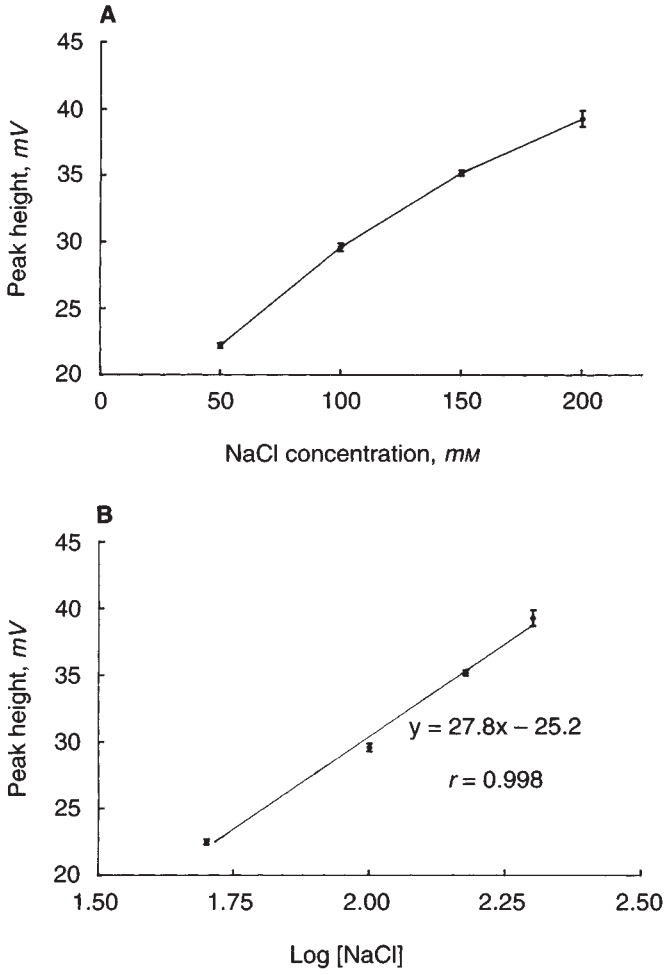


**Fig. 2.** *Standard curve for sodium.* **A.** Linear plot of the data. **B.** Semi-logarithmic plot of the data. The pipet volume was 10.9 nl. Values of the mean ± SD for three samples were: 50 mM, 22.1 ± 0.2 mV; 100 mM, 29.9 ± 0.3 mV; 150 mM, 35.2 ± 0.2 mV; 200 mM, 39.3 ± 0.2 mV. All solutions contained 100 mM $MgCl_2$.

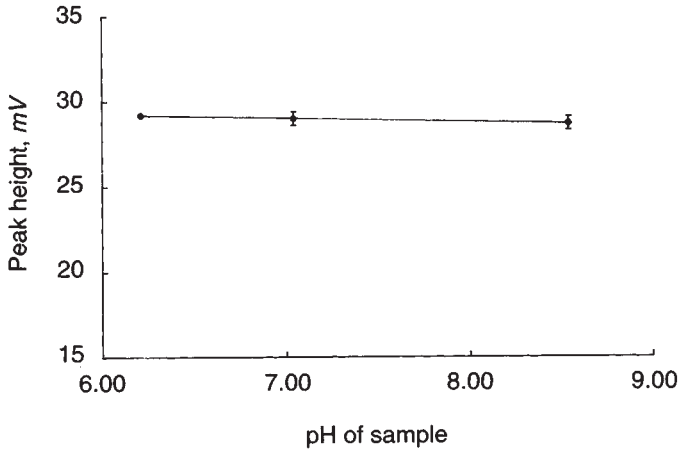


**Fig. 3.** *Effect of pH on response to 100 mM NaCl.* The pH of solutions containing 100 mM NaCl, 100 mM $MgCl_2$ and 20 mM HEPES was adjusted to 6.21, 7.04 and 8.54. Mean values ± SD are shown.

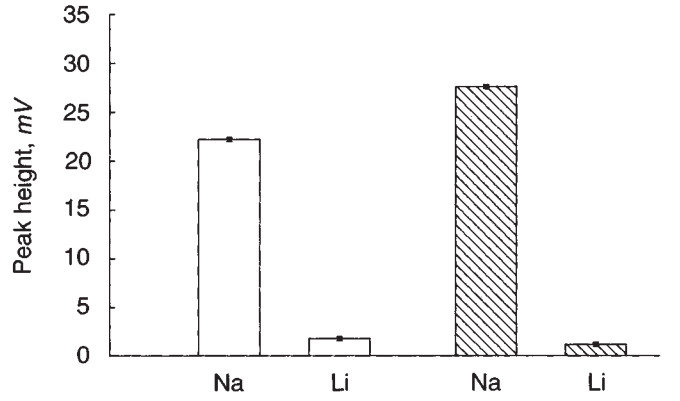


**Fig. 4.** *Response to 50 mM LiCl compared to 50 mM NaCl before and after treating the detector with NaOH and HCl.* Both solutions contained 100 mM $MgCl_2$. Mean values ± SD are shown.

**Table 1.** Response to 100 mM NaCl in the presence of $NH_4Cl$, KCl or $CaCl_2$

| Na Conc. *mM* | XCl *mM* | Peak height *mV* | *N* |
|---|---|---|---|
| 100 | — | 29.8 ± 0.4 | (3) |
| 100 | $NH_4Cl$ (5) | 29.9 ± 0.4 | (3) |
| 100 | — | 29.5 ± 0.4 | (3) |
| 100 | KCl (5) | 29.6 ± 0.2 | (3) |
| 100 | — | 29.0 ± 0.1 | (3) |
| 100 | $CaCl_2$ (5) | 29.2 ± 0.4 | (3) |

Peak heights are reported as mean ± SD. All solutions contained 100 mM $MgCl_2$. The different chloride salts were tested on different days.

mM $CaCl_2$ was measured. No significant difference in response was found (Table 1).

Changes in the pH of the sample may also be of some concern, since it is known that many nephron segments which transport sodium also transport protons or bicarbonate [14, 15]. Figure 3 shows the effect of varying pH on the response to 100 mM NaCl. Samples contained 100 mM NaCl and 20 mM N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonate] (HEPES) and pH was adjusted to 6.21, 7.04 and 8.54, respectively. The mean peak heights for each pH were 29.1 ± 0.2 mV; 28.9 ± 0.4 mV; and 28.7 ± 0.4 mV. Thus, changing the pH over the range from 6.21 to 8.54 had no significant effect on the response to 100 mM NaCl. The fact that 100 mM NaCl alone yielded a mean peak height of 29.0 ± 0.1 mV on the day the pH studies were performed indicates that anions other than chloride have no effect on peak height.

To further study the effect of pH, and to show that different anions do not interfere with the measurement of sodium, the response to 100 mM NaCl was compared to the response to 75 mM NaCl plus 25 mM $NaHCO_3$. No significant difference between the two solutions was found (difference 0.2 ± 0.2 mV).

Finally, the response to 50 mM LiCl was tested, since lithium is the ion most likely to interfere with the measurement of sodium given the similarity in size: this is reflected by the fact that many transport proteins which translocate sodium will also translocate lithium [16–18]. Figure 4 shows the relative peak heights for samples containing either 50 mM LiCl or the same

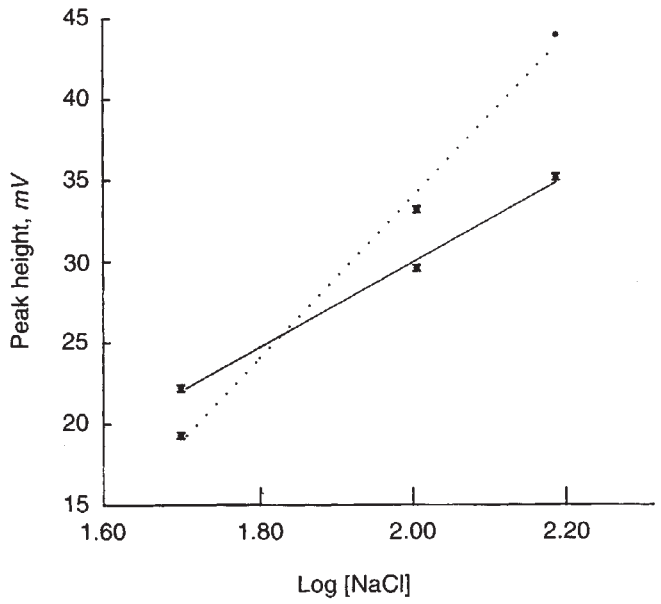


**Fig. 5.** *Comparison of the responses to 50, 100 and 150 mM NaCl before and after incubating the detector in NaOH and HCl.* Dotted line represents data collected before treatment; solid line represents data collected after treatment.

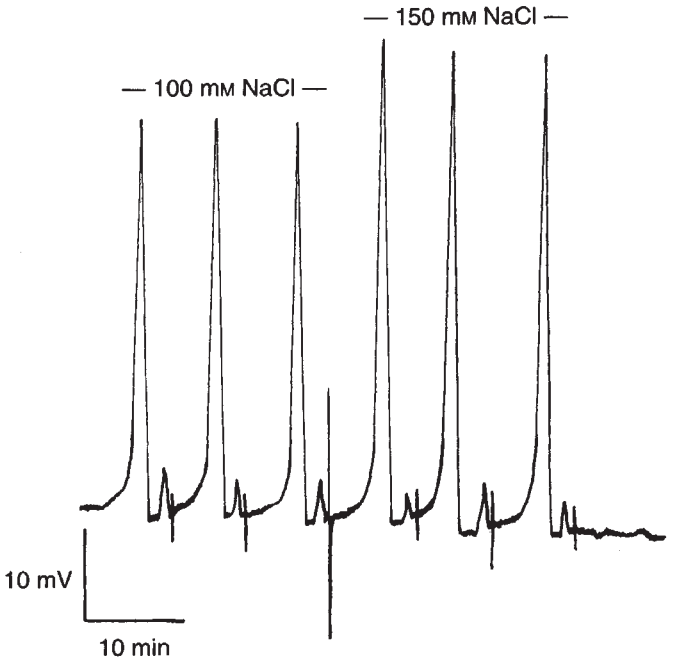


**Fig. 6.** *Typical results obtained with the potentiometric device.* The responses to 10.7 nl of 100 mM and 150 mM NaCl are shown. The disturbance in the baseline and the small peak before the main peak are injection artifacts.

concentration of NaCl. The mean peak height for LiCl was 1.8 ± 0.2 mV, while that for sodium was 22.1 ± 0.2 mV. Thus, lithium may be a significant interfering ion.

The supplier of the sodium-sensitive glass reported that brief exposure of the glass to NaOH and HCl would improve its selectivity and sensitivity. To determine whether greater selectivity and peak resolution might be possible over the physiological range of sodium concentrations, we incubated the sodium-sensitive glass detector first with 100 mM NaOH and then with 100 mM HCl for 10 minutes each before use. NaCl concentrations of 50, 100 and 150 mM were examined, since they cover the physiological range for sodium. After such maneuvers, the mean peak height ± SD for three samples of 50 mM NaCl was 19.4 ± 0.2 mV; for 100 mM NaCl it was 33.2 ± 0.2 mV, while for 150 mM NaCl it was 44.0 ± 0.1 mV. Figure 5 compares the responses to 50, 100 and 150 mM NaCl before and after pretreatment of the detector with NaOH and HCl to increase sensitivity and selectivity. In this particular example the slope increased from 27.8 to 51.2 mV per decade increase in sodium concentration. After treatment, the range of slopes on different days was 51 to 58 mV per decade.

Figure 6 shows typical data obtained from the device after treatment of the sodium-sensitive glass. The response to 10.7 nl of 100 and 150 mM NaCl is shown. The baseline is extremely stable and the peaks are sharp. There is, however, a small peak prior to the main peak and a disturbance in the baseline which are injection artifacts. The magnitude of these artifacts depends on the quality of the shielding.

To further investigate the selectivity of the potentiometric detector after treatment with NaOH and HCl, the response to 100 mM NaCl was compared to the response to 100 mM NaCl plus 25 mM KCl or $NH_4Cl$, after which the response to 50 mM LiCl was again measured. The response to 100 mM NaCl alone was 43.6 ± 0.7 mV, while that to 100 mM NaCl plus 25 mM KCl was 43.4 ± 0.8 mV. In a separate study, the response to 100 mM NaCl was 46.6 ± 0.4 mV and that to 100 mM NaCl plus 25 mM $NH_4Cl$ was 47.1 ± 0.4 mV. Thus, even at concentrations 25 mM, $K^+$ and $NH_4^+$ did not significantly interfere with the response to 100 mM NaCl.

The response to 50 mM LiCl after treatment of the detector with NaOH and HCl was compared to the response before treatment. Prior to treatment, the response to 50 mM NaCl was 22.1 ± 0.2 mV, while that to the same concentration of LiCl was 1.8 ± 0.2 mV. After treating the detector, the response to 50 mM NaCl was 27.6 ± 0.2 mV, whereas the response to LiCl was 1.2 ± 0.2 mV (Fig. 4). Thus, the selectivity for $Na^+$ over $Li^+$ improved.

The response to N-methyl D-glucamine chloride was also tested, since this salt is often used to replace sodium in physiological experiments. The mean response to 150 mM N-methyl D-glucamine chloride was 0.1 ± 0.1 mV.

## Discussion

This report describes a new instrument designed to measure picomole amounts of sodium. This apparatus was developed so that sodium fluxes could easily be determined in isolated, perfused renal tubule experiments where the sodium concentration generally ranges from 100 to 150 mM in experiments involving the collecting duct and 50 to 150 mM in experiments involving the thick ascending limb of the loop of Henle, while the sample size ranges from 5 to 10 nanoliters.

Methods of determining sodium flux can be divided into two categories: (1) use of radioactive isotopes, and (2) use of spectroscopy to analyze sodium content of collected samples.

The latter has been limited to atomic absorption spectrophotometry [1, 2], electron probe analysis [3–6], helium-glow photometry [7, 8], and colorimetry using chromogenic macrocyclic ionophores [9]. The instrument described here has many advantages over previous techniques. First, it is primarily constructed from relatively inexpensive and commercially available components, except for the sodium-sensitive glass detector and injection port which can easily be fabricated on a standard microforge. For instance, the most expensive component, the electrometer, currently sells for $1,400.00. Second, there is no need to take special care in handling the samples as with radioactive isotopes or atomic absorption spectrophotometry, nor do the samples have to be specially prepared as with atomic absorption spectrophotometry and electron probe analysis. Additionally, extensive training is not required as with helium-glow photometry. Finally, the sodium content of a sample can be measured in as little as four minutes which gives this technique a distinct advantage over all other methods.

The potentiometric response of the detector to changes in sodium concentration was logarithmic, as predicted from the Nernst equation (Figs. 2 and 5). However, over a small range of sodium concentrations such as 100 to 150 mM only a small error will be introduced if the response of the detector is assumed to be linear.

By incubating the sodium-sensitive glass detector with 100 mM NaOH for 10 minutes and washing it with 100 mM HCl before use, both resolution and selectivity of the detector improved. Although the relationship between peak height and NaCl concentration was logarithmic, the average resolution from 50 to 100 mM and from 100 to 150 mM, NaCl was calculated assuming a linear response for simplicity. This assumption will introduce only a small error in the calculation of resolution over this limited range. Resolution was defined as twice the average standard deviation for measurements of 50, 100 and 150 mM NaCl, divided by the slope of the line relating peak height and concentration between 50 and 100 mM NaCl, and again between 100 and 150 mM NaCl. The average standard deviation was 0.14 mV, while the slope between 50 and 100 mM NaCl was 0.28 mV/mM and that between 100 and 150 mM NaCl was 0.22 mV/mM. Consequently, resolution between 50 and 100 mM was approximately 1.0 mM, while that for 100 to 150 mM NaCl was approximately 1.3 mM.

The resolution of other methods is both better (atomic absorption spectrophotometry) and worse (chromogenic macrocyclic ionophores) than the potentiometric instrument described here. However, with an average resolution of approximately 1 to 2 mM using a 10.9 nl sample (11 to 22 pmol) in the 100 to 150 mM (1090 to 1635 pmol) range, this device is capable of resolving differences in sodium concentration in isolated tubule perfusion experiments. This is especially true as the sodium concentration falls, since resolution improves due to the logarithmic response of the detector with decreasing concentration.

The sensitivity of the device did not change significantly over the two to three hours in which the results obtained on a given day were collected. If the peak height of a sample with a known concentration of sodium changed significantly, this indicated that a bubble was forming in the flowing stream. The frequency of this problem can be reduced with careful preparation of the reservoir solution. However, as with all ultramicro flow-through devices, a standard should be used at regular intervals to assure that there has not been a significant change in sensitivity.

Interference by cations commonly encountered in physiological solutions does not appear to be an important concern when measuring sodium content with the potentiometric instrument. Samples containing potassium, calcium, or ammonium in addition to sodium produced peaks that were indistinguishable from those containing sodium alone. Additionally, changes in pH from 6.21 to 8.54 had no significant effect on peak height. The device also detected lithium, which is not normally found in physiological solutions but is often used as a substitute for sodium. Given the low selectivity of sodium over lithium (approximately 20 to 1), it is unlikely that sodium concentration could be reliably measured in solutions containing lithium. N-methyl D-glucamine, another sodium substitute, did not elicit a significant response compared to sodium. The selectivity for sodium over N-methyl D-glucamine was approximately 300 to 1.

There also was no interference by anions other than chloride. Addition of 20 mM HEPES, half of which would be in the anionic form at its pK, had no significant effect. Additionally, substituting 25 mM bicarbonate for chloride in a solution with 100 mM Na had no significant effect on the peak.

The absolute response of the device varied from 51 to 58 mV per decade increase in sodium after treatment with NaOH and HCl. It is not clear why the slope is sometimes less than 58 mV per decade, nor why the slope varies. The answers to both questions are not entirely clear; however, this problem is often encountered when using ion-sensitive electrodes. One possible explanation may be that small variations in pump speed from day to day alter the rate at which the sample is swept along to encounter the sodium-sensitive glass. This in turn would cause dilution of the sample by the flowing solution. Whatever the explanation, this variability does not seriously affect the resolving power of the device, since the smallest slope obtained was used to calculate the resolution.

*Summary*

This report describes a new potentiometric instrument designed to measure picomole quantities of sodium based on a sodium-sensitive glass detector. The instrument is highly specific for sodium, with a resolution of approximately 1.2 mM in the 50 to 150 mM range utilizing a 10.9 nl sample. Samples can be injected approximately once every four minutes; consequently, sodium concentration can be measured in "real time" during most physiological experiments. This device costs far less and is simpler to use than currently available commercial instruments used to measure picomole quantities of sodium. Given these attributes, this instrument represents a significant improvement over existing technologies.

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